Dept. of Vet. Medicine,
Faculty of Vet. Med., Assiut University,
Head of Dept. Prof. Dr. M.F. Raghib.

MOTILE AEROMONAS SEPTICAEMIA (MAS) IN MORMYRUS KANNUME AT ASSIUT GOVERNORATE

(With 1 Table & 3 Figs.)

By

SH.M. AHMED; A.M. ZAITOUN and H.S. ALI

(Received at 5/12/1990)

تسمم الايروموناس الدموى في أسماك البوبيز
في محافظة أسيوط

شعبان أحمد ، أحمد زيتون ، حلمي صديق

أجرى هذا البحث على سمك البوبيز لمعرفة دور ميكروب الايروموناس . وقد شمل البحث مائة سمكة منهم عشرون سمكه ظهر عليها نقطة نزيفية في اماكن مختلفة من الجسم وكذلك تعفن في الزعانف وقروح على الجسم. وقد تم عزل عشرة عترات من الايروموناس من الأعضاء الداخلية والجسم وصنفت الى عترة واحدة وهي ايروموناس هيدروفيلا وقد تم دراسة ضراوة العترات المعزولة بالطرق المختلفة.

SUMMARY

The present investigation reveals the pathogenicity Aeromonas hydrophila to Morymrus Kannume. One hundred Mormyrus Kannume fish were the subjected of this work out of them twenty fish showed haemorrhages on different part of the body, fins rot and ulcerative lesions.

Ten isolates of Aeromonas hydrophila were recovered from internal organs, ascitic fluid and skin. Acriflavine agglutination; stability after boiling and pathogenicity tests were applied to detect the virulence of the isolated strains.

INTRODUCTION

Motile Aeromonas species are widely distributed in nature and are an ubiquitous component of the aquatic environments, (HAZEN, et al. 1978; NEILSON, 1978 and CIPRIANO, et al. 1984).

Motile Aeromonas species were identified as a significant pathogen of fish, cold blooded animals and human (BULLOCK, 1961; SHOTTS, et al. 1972 and AUSTIN

and AUSTIN, 1987) and DAVIS, et al. 1978) respectively. Moreover, POST (1983) reported that all fresh and salt water fish are susceptible to motile Aeromonas septicaemia.

Many investigators recorded MAS in different species of fish; carp, catfish, bass, ayu, perch and tilapia (AFANAS'YEV, et al. 1975; DE FIGUERIREDO and PLUMB REF, 1977; HAZEN, et al. 1978; JO and OHNISHI, 1980; MICHEL, 1981 and AHMED, 1982).

The aim of the present work was planned to study the role played by A.hydrophila in naturally and expeimentally infected Mormyrus Kannume.

MATERIAL and METHODS

One hundred Mormyrus Kannume collected from fresh water cannal, (El-Ebrahimia Cannal, Assiut Governorate) were used in this work.

Out of these, twenty fishs suspected to be infected with MAS were brought alive into Laboratory as quickly as possible under aseptic condition.

Experimental procedure:

Mormyrus Kannume (40-45 gm) were collected from the River Nile (Assiut) and brought to the Laboratory. Fish were kept in glass aquaria for three weeks for adaptation and for further pathogencity tests. Random Samples were subjected to bacteriological and parasitological examinations to insure fish pathogen free.

Clinical and Post mortem examinatins:

Clinical and Post mortem examinations were carried out and recorded as described by AUSTIN and AUSTIN (1987).

Bacteriological examination:

Samples from skin, liver, kidney and ascitic fluid of infected fish were cultured on trypticase soya agar & blood agar media and incubated at 22°C for 48 hours. The isolates were identified morphologically and biochemically according to the methods described by COWAN and STEELS (1974); ALLEN, et al. (1983) and POPOFF (1984).

Acriflavine agglutination test and stability after boiling were conducted to detect the virulance of isolated A.hydrophila as reported by MITTAL, et al. (1980).

Pathogenicity test:

Forty four fish of Mormyrus Kannume were divided into eleven groups (each of four). Ten groups were inoculated intraperitoneally each with one of the isolated

10 strains of A.hydrophila. The inoculum was composed of 0.5 ml broth culture containing $2x10^7$ living bacterial cell. The eleventh control group was inoculated with 0.5 ml Sterile broth. Assay of pathogenicity test was conducted at 19±1°C with daily observation to detect the morbidity and mortality rates for two weeks.

RESULTS

Clinical and Postmortem findings:

Signs of infected fish included a wide spread peticheal haemorrhages which was so evident in eye and bases of the dorsal, pectoral and pelvic fins associated with fin rot (Fig. 1 & 2).

Diffuse haemorrhages with sticky gelatinous material, greyish white in colour at the caudal portion of the body, especially tail fins, were also noticed (Fig. 3). Some infected fish showed haemorrhagic and protruded anal orifice with white central ulcerative lesion surrounded by clear narrow haemorrhagic zone (Fig. 2). All internal organs particularly ovaries were congested, little amount of yellowish serous fluid was found in the body cavity.

Bacteriological examination:

Primary isolation revealed that 10 isolates were recovered from 20 infected fish. The isolates were identified as A.hydrophila.

Acriflavine agglutination test and Stability after boiling:

Seven out of ten isolated strains did not agglutinate in acriflavine and settled down after boiling, these were the Highly virulence strains. The other strains agglutinated in acriflavine and did not settle down after boiling (Table 1).

Pathogenicity test:

Inoculated fish with strains No. 1, 2, 4, 5, 6, 8 and 9 died within five days showing the same clinical signs and post mortem findings described previously; however no ulcerative lesions were noticed. The other inoculated fish with strains No. 3, 7 and 10 gave negative result (Table 1). A.hydrophila was isolated from the internal organs particularly liver and kidney of the dead infected fish.

DISCUSSION

Motile Aeromonas Septicaemia has a world wide distribution in many countries. Many authors reported that A.hydrophila, is considered as either a primary agent

or secondary to viral infection or parasitic infestation. Moreover, unfavourable environmental conditions play a role in the incidence of the infection. All types of freshwater fish are susceptible to infection (BULLOCK, 1961; OTTE, 1963; BULLOCK, et al. 1971; RICHARDS, 1977; POST, 1983 and ANDREWS, et al. (1988).

From the obtained results, 10 strains of A.hydrophila were isolated from 20 naturally infected fish (Mormyrus kannume). All the isolated strains were tested experimentally and proved that, A.hydrophila could be considered as the primary cause of MAS in Mormyrus Kannume. To the best of our knowledge and from the available literature it appears most likely that this work is the first record of MAS infection in Mormyrus Kannume.

Only seven out ten isolated strains did not agglutinate in acriflavine, settled down after boiling and killed all the incoculated fish within 5 days (Table 1). These results revealed that, acriflavine agglutination, stability after boiling and pathogenicity tests can be used to detect the virulence of A.hydrophila, a similar observation had been previouly recorded by MITTAL, et al. (1980).

REFERENCES

Afanas'yev, V.I.; Slynko, L. and Kolot, G.S. (1975): Features of electrolyte metabolism in healthy carp (Cyprinus capio) and those infected with red-spot disease. J. of Icthyology, 15: 514-518.

Ahmed, M.Sh. (1982): Natural and Experimental studies of Motile Aeromonas Septicaemia in freshwater fish (Tilapia nilotica) M.V.Sc. Thesis. Fac. of Vet. Med., Assiut University, Egypt.

Allen, D.A.; Austin, B. and Colwell, R.R. (1983): Numerical taxonomy of bacterial isolates associated with a freshwater fishery. J. of general Microbiol., 129: 2043-2062.

Andrews, C.; Exell, A. and Carrington, N. (1988): The Manual of fish Health. Salamander Books Limited. London, New York. 1st ed.

Austin, B. and Austin, D.A. (1987): Bacterial fish pathogens, diseases in farmed and wildfish. Ellis Horwood Limited, England. 1st ed.

Bullock, G.L. (1961): The identification and separation of Aeromonas liquefaciens from Pseudomonas fluorescens and related organisms occuring in diseased fish. Appl. Microbiol., 9: 587-590.

Bullock, G.L.; D.A. Conroy and Snieszko, S.F. (1971): Bacterial disease of fishes Diseases of fishes, Book 2, A.S.F., Snieszko and H.R. Axelrod (Editors) T.F.H. Publications, Jeraycity, N.T. 1st ed.

Cipriano, R.C.; Bullock, G.L. and Pyle, S.W. (1984): Aeromonas hydrophila and motile Aeromonas Septicaemia of fish Disease Leaflet 68 United states Department of the Interior fish and Wildlife service. Division of Fishery Research Washington, D.C. 20240.

Cowan, S.T. and Steels, K.J. (1974): Manual for the identification of Medical bacteria. Cambridge University, Cambridge. 1st ed.

Davis, W.A.; Kane, K.J. and Garagusi, V.F. (1978): Human Aeromonas infection, J. of Medicine, 57, 3: 267-277.

De Figueiredo, J. and Plumb, J.A. (1977): Virulence of different isolates of Aeromonas hydrophila in channel catfish Aguaculture 11: 349-354.

Hazen, T.C.; Fliermans, C.B.; Hirsch, R.P. and Esch, G.H. (1978): Prevalence and distribution of Aeromonas hydrophila in the United States. Appl. Environ, Microbiol., 36: 731-738.

Jo, Y. and Ohnishi, K. (1980): Aeromonas hydrophila isolated from cultured ayu. Fish path., 15: 85-89.

Michel, C. (1981): A bacterial disease of perch (Perca fluviatilis L.) in an Alpine Lake: isolation and preliminary study of the causative organism. J. of Wildlife Disease, 14: 15-19.

Mittal, K.R.; Lalonde, G.; Leblanc, D.; Olivier, D. and Lallier, R. (1980): Aeromonas hydrophila in rainbow trout. relation between virulence and surface characteristics. (1980). Can. J. Microbiol., 26: 1501-1503.

Neilson, A.H. (1978): The occurence of Aeromonads in activated sludge: isolation of Aeromonas sobria and its possible confusion with Escherichia coli. J. Appl. Bacteriol., 44: 259-264.

Otte, E. (1963): Die heutigen Ansichten Uber die Atiologie der infektionen Bauchwassersucht der karpfen. Wien. Tierartzl. Monatsschr. 50 (11): 996-1005.

Popoff, M. (1984): Genus III. Aeromonas Kluyver and Van Niel 1963. In Krieg, N.R. (ed) Bergey's Manual of systematic Bacteriology Vol. 1, Baltimore Williams and Wilkins.

Post, W.G. (1983): Textbook of fish health TFH Publications, Inc. Ltd.

Richards, R. (1977): Diseases of aquarium fish -3: Disease of the internal Organs Vet. Record 101: 149-150.

Shotts, B.E.JR.; Gaines, J.L.; Martin, JR.L. and Prestwood, A.K. (1972): Aeromonas induced deaths among fish and reptiles in an eutrophic inland lake. J. Am. Vet. Med., Assoc., 161: 603-607.

Table (1)
Different tets used for the detection of virulence of A. hydrophilia

No. of group	No. of isolate	Acriflavine agglutination test	Appearance after boiling	No. of dead fish after inoculation		No. of survivors after 15 days
				1-2 days	3-5 days	
1	1	-	+	4	-	-
2	2	-	+	2	2	-
3	3	+	-	-	-	4
4	4	-	-	3	1	-
5	5	-	+	4	-	-
6	6	-	+	2	2	-
7	7	+	-	-	1	3
8	8	-	+	3	1	-
9	9	-	+	4	-	-
10	10	+	-	-	-	4
11	Sterile Broth used as control.					4

- No agglutination in acriflavine or settling down after boiling.
+ agglutination in acriflavine and no settling down after boiling.

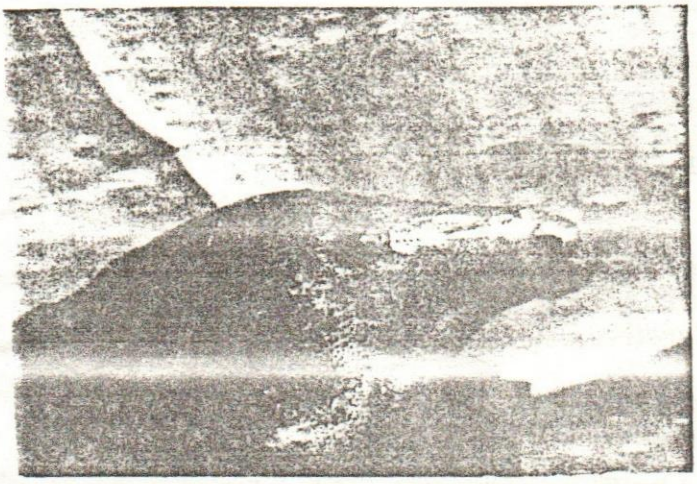

Fig. (1)
Haemorrhage in the eye.

Fig. (2)
Ulecerative Lesions Surrounded by haemorrhage

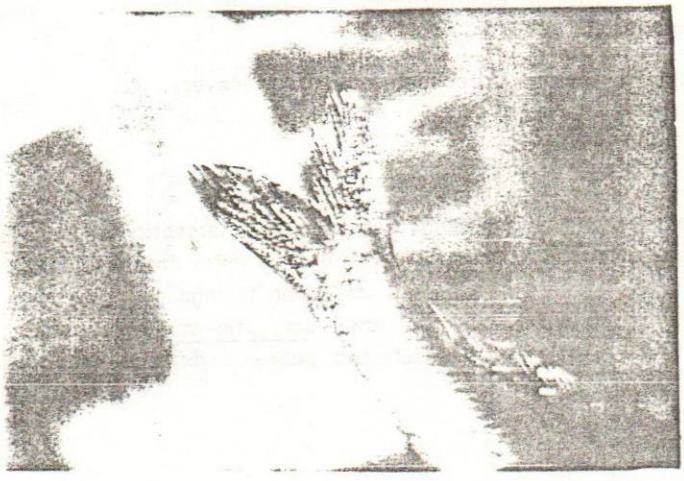

Fig. (3)
Tail fins suffered from haemorrhage and rot.